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THE CHROMOPHORE OF VIOMYCIN

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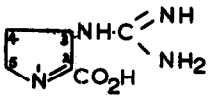
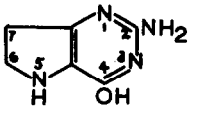
Acid hydrolysis of the strongly basic tuberculostatic antibiotic, viomycin[1,2] is known to yield L-serine, L-αβ-diaminopropionic acid, L-β-lysine, carbon dioxide, ammonia, urea and a guanido compound. In a recent paper,[3] Dyer and his colleagues suggested that the guanido compound, which was named viomycidine, was 3-guanido-1-pyrroline-2-carboxylic acid (I) on the grounds of its physical properties and a number of degradative reactions, including nitric acid oxidation to guanidine, and alkaline hydrolysis to pyrrole-2-carboxylic acid, 2-aminopyrimidine, and glycine (experimental details not yet available). We have also isolated viomycidine from viomycin and characterised it as its p-hydroxybenzeneazosulphonate[2] and dipicrate, m.p. 148–150° (Found: C, 33.8; H, 2.75; N, 22.3. $C_6H_{10}N_4O_2 \cdot (C_6H_3N_3O_7)_2$ requires C, 34.2; H, 2.55; N, 22.3%). We agree with the formulation of viomycidine as (I) on the following evidence: it shows pKa values 1.5, 5.7, and 12.4 (in water) and it contains a carboxyl and a monosubstituted guanido substituent (on the basis of infrared spectrum cf.[4]) and gives positive ninhydrin and Sakaguchi tests. It shows no max. above 215 mμ in the ultraviolet, and is hydrogenated in presence of palladium-charcoal with absorption of one mole. of hydrogen. Oxidation with aqueous permanganate gives guanidine and glycine. The

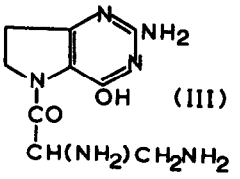
nuclear magnetic resonance spectrum of viomycidine in deuterium oxide shows max. at 7.68 (triplet; 2), 5.87 (multiplet; 2) and 4.98 (triplet; 1) (numbers of protons in parenthesis), assigned, from a consideration of model systems, to the protons at C_4, C_5 and C_3 respectively. As there are no absorptions to low field of 4.98τ and as the nature of the 7.68 and 5.87 absorptions were unaltered when the spectrum of an aqueous solution was measured, the double bond must be in the Δ^1-position.



(I)



(II)



(III)

Viomycin itself shows ultraviolet absorption at 268 mμ ($E^{1\%}_{1cm.}$ 320) at pH 1, and at 290 mμ ($E^{1\%}_{1\ cm.}$ 210) at pH 10. In order to explain this, we suggest that viomycidine is present in the antibiotic as the cyclised structure (II), a derivative of 6,7-dihydro-5H-pyrrolo[3,2-d]pyrimidine, and it is of interest that a 7H-pyrrolo [2,3-d]pyrimidine structure has recently been reported for the antibiotic tubercidin[5] from Streptomyces tubercidicus. The spectrum of viomycin resembles that of 2,5-diamino-4-hydroxypyrimidine[6] and cleavage of the pyrimidine ring in 5,6-fused bicyclic 2-amino-4-hydroxypyrimidines under acid conditions has been described[e.g.7] as well as the ring opening of 5,6-dihydro-2-amino-4-hydroxypyrimidine to β-guanidopropionic acid.[8]

The isolation and properties of a partial hydrolysis product, peptide A, lends further support to the above suggestions. Peptide A forms a crystalline tripicrate, m.p. 174-176° [Found: C, 35.1:

H, 2.75; N, 22.6. $C_9H_{14}N_6O_2 \cdot (C_6H_3N_3O_7)_3$ requires C, 35.1; H, 2.55; N, 22.7%. The peptide gave positive ninhydrin and Sakaguchi reactions, it showed pKa 9.9 and 11.2, and was not hydrogenated in presence of palladium-carbon. The light absorption properties of peptide A were similar to those of viomycin, viz. absorption at 275 mμ (ε 5100) at pH 1 and at 295 mμ (ε 3600) at pH 10. Hydrolysis of peptide A with boiling 12N hydrochloric acid gave αβ-diamino-propionic acid and viomycidine in a 1:1 molar ratio. These were separated over Dowex 50W x 8 and characterised as the monohydrochloride and p-hydroxyazobenzenesulphonate respectively. Treatment of peptide A with an excess of 2,4-dinitrofluorobenzene gave a derivative which on hydrolysis yielded αβ-bis(2,4-dinitrophenylamino)propionic acid. Oxidation of peptide A with dilute aqueous permanganate gave guanidine and a peptide which was hydrolysed to give αβ-diaminopropionic acid. On this evidence structure (III) is postulated for peptide A, the chromophore of which is also contained in the antibiotic itself. Work on the structure of other dipeptides isolated from degradations of viomycin is in progress.

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